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Efficient production of 2,3-butanediol by recombinant *Saccharomyces cerevisiae* through modulation of gene expression by cocktail δ-integration

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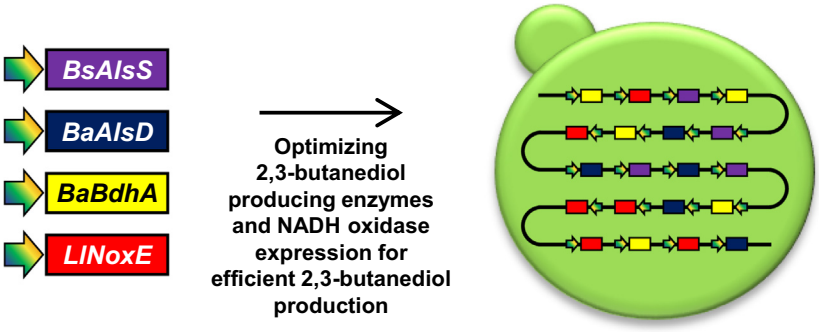
HIGHLIGHTS

- 2,3-Butanediol (BDO) is useful metabolites produced by microorganisms.
- BDO production by *Saccharomyces cerevisiae* was examined.
- Expression of 4 genes was optimized by cocktail δ-integration strategy.
- BDO productivity was improved by cocktail δ-integration strategy.
- The highest BDO production rate and yield were achieved in fed-batch cultivation.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, the expression of 4 genes encoding α-acetolactate synthase, α-acetolactate decarboxylase, 2,3-butanediol dehydrogenase, and NADH oxidase was modulated using a previously developed cocktail δ-integration strategy. The resultant strain, YPH499/dPdAdG/BD6-10, was used in a fed-batch cultivation for the production of 2,3-butanediol. The concentration, production rate, and yield obtained were 80.0 g/L, 4.00 g/L/h, and 41.7%, respectively. The production rate and yield of the compound obtained are higher for this strain compared to reports published for *Saccharomyces cerevisiae* so far. The cocktail δ-integration strategy allows for modulation of multiple gene expression, without the exact knowledge of rate-limiting steps, and therefore, could be used as a promising strategy for the production of bio-based chemicals in recombinant *S. cerevisiae*.

1. Introduction

The use of renewable feedstock such as cellulose and hemicellulose, and their constituent sugars glucose and xylose, for the production of bio-based chemicals by engineering metabolic pathways in various kinds of microorganisms has recently gained prominence because of the environmental problems associated with the combustion of fossil fuels (Jullesson et al., 2015). Among the host microorganisms used for metabolic engineering, the yeast *Saccharomyces cerevisiae* is particularly attractive due to its safety and convenience of use. *S. cerevisiae* is nonpathogenic, classified as a "generally regarded as safe" organism, and has long been used to produce organic compounds like ethanol (Ostergaard et al.,

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2000). Thus, the fermentation and processing technology for large-scale production is well established for *S. cerevisiae*.

2,3-Butanediol is one of the most useful metabolites produced by microorganisms, as both the compound and its derivatives can be used as fuels, resins, solvents, food additives, cosmetics, drugs, and fumigants (Bialkowska, 2016; Molitor et al., 2016). Thus, it has several applications in the chemical, food, cosmetic, and pharmaceutical industry, as well as in agriculture (Kim et al., 2016). The most efficient 2,3-butanediol-producing bacteria are *Klebsiella pneumoniae* and *Klebsiella oxytoca* (Bialkowska, 2016; Kumar et al., 2016). However, these bacteria are pathogenic, thereby restricting large-scale production because of strict safety regulations (Bialkowska, 2016; Celinska and Grajek, 2009). Although, alternative non-pathogenic 2,3-butanediol producers such as *Bacillus subtilis* (Biswas et al., 2012; Fu et al., 2016), *Bacillus amyloliquefaciens* (Sikora et al., 2016; Yang et al., 2011), and *Lactococcus lactis* (Gaspar et al., 2011; Kandasamy et al., 2016) have also been reported, the productivity does not fulfil industrial requirements. Thus, there is a need to search for an efficient non-pathogenic microorganism with the ability to produce 2,3-butanediol.

Non-pathogenic *S. cerevisiae* is a promising host microorganism that could be used for the production of 2,3-butanediol. There have been several reports about the production of the organic compound with metabolically engineered *S. cerevisiae* harboring the bacterial 2,3-butanediol biosynthetic pathway (Fig. 1) (Kim et al., 2013, 2016, 2017; Kim and Hahn, 2015; Ng et al., 2012). Expression of a *B. subtilis* α-acetolactate synthase and α-acetolactate decarboxylase and over-expression of the endogenous 2,3-butanediol dehydrogenase could lead to the conversion of pyruvate into optically pure (2R,3R)-butanediol by the Pdc-deficient *S. cerevisiae* (Kim et al., 2013). High yielding production, however, causes a redox imbalance (Kim and Hahn, 2015). This is due to the production of an extra molecule of NADH in a chemical reaction, where one molecule of glucose is converted into one molecule of 2,3-butanediol (Fig. 1). To address this issue, NADH oxidase from *L. lactis* was co-expressed along with the 2,3-butanediol producing enzymes in *S. cerevisiae* (Kim and Hahn, 2015; Kim et al., 2016). However, the productivity in this metabolically engineered *S. cerevisiae* is still low when compared to that obtained using the aforementioned pathogenic bacteria (Kim et al., 2012; Ma et al., 2009).

Recently, the cocktail δ-integration strategy has been developed, which involves the simultaneous integration of various multi-copy genes via δ-integration, followed by the selection of desirable transformants in *S. cerevisiae*. Using this strategy, expression of genes encoding cellulase (Yamada et al., 2010a,2011; Liu et al., 2017), those related to glycolysis (Yamada et al., 2017a), and genes involved in lactate production (Yamada et al., 2017b), and xylose assimilation (Kato et al., 2013) in *S. cerevisiae* were optimized. The strategy allows for modulation of more than ten different kinds of gene expression simultaneously, without the exact knowledge of the rate-limiting steps. Hence, this strategy could be useful for optimizing complicated metabolic pathway including the redox imbalance issue.

In this study, genes encoding α-acetolactate synthase from *B. subtilis* (*BsAlsS*), α-acetolactate decarboxylase (*BaAlsD*) and butanediol dehydrogenase (*BaBdhA*) from *B. amyloliquefaciens*, and NADH oxidase from *L. lactis* (*LlNoxE*) were expressed in *S. cerevisiae* deficient in genes that code for alcohol dehydrogenase (*ADH1*), pyruvate decarboxylase (*PDC1*), and glycerol-3-phosphate dehydrogenase (*GPD1*) using the cocktail δ-integration strategy. Fed-batch cultivation was then carried out to produce 2,3-butanediol using the modified *S. cerevisiae* strain.

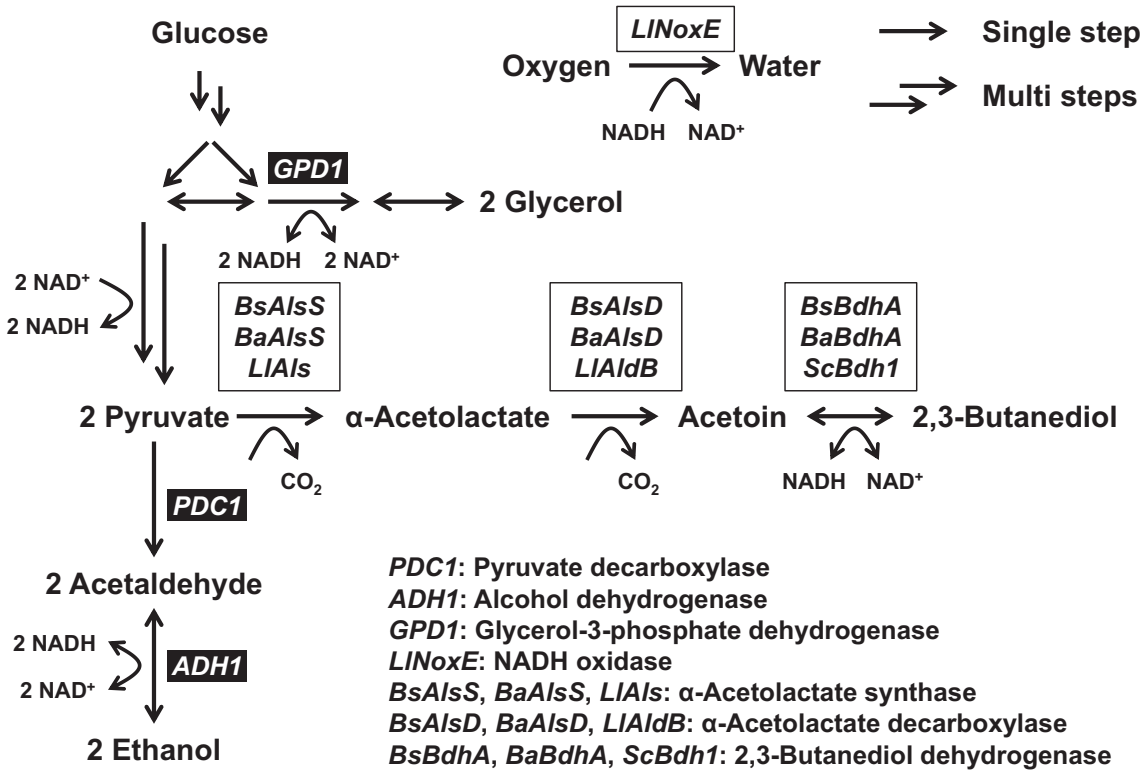

Fig. 1. Metabolic pathway for 2,3-butanediol production. Directional arrows and bidirectional arrows represent irreversible and reversible reactions, respectively. Genes indicated in the white boxes were overexpressed, and genes in the black boxes were deleted in this study.

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